

A Cytological Study of *Saussurea* Subgenus *Eriocoryne* (Asteraceae) from the Nepal Himalaya

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Somatic chromosome numbers and karyotypes of eight species of *Saussurea* subgenus *Eriocoryne* collected from the Nepal Himalaya are reported. The karyotypes of all but one species were analyzed for the first time. The chromosome numbers of *Saussurea laminamaensis*, *S. bhutkesh* and *S. kanaii* were reported for the first time. The chromosome numbers were $2n = 32$ and 36 . The chromosome number $2n = 36$ was found in *S. gossipiphora*, *S. laminamaensis*, *S. nishiokae*, *S. tridactyla* and *S. bhutkesh*; $2n = 32$ was found in *S. simpsoniana*, *S. topkegolensis* and *S. kanaii*. All of these are considered to be diploid, but the basic chromosome numbers, $x = 16$ and 18 , differ among the species. These findings suggest an evolutionary trend toward the formation of aneuploids at the diploid level during species diversification in the Himalayas. The chromosomes were gradually reduced from the longest to the shortest and had median, submedian, subterminal, and terminal centromeres.

Key words: Asteraceae, chromosome number, karyotype, Nepal Himalaya, *Saussurea*.

Saussurea DC. (Asteraceae), distributed mainly in temperate and subarctic regions of Eurasia, and consisting of about 400 species, is subdivided into six subgenera (Lipschitz 1979). Thirty four species have been described in subgenus *Eriocoryne*, which is characterized by the unbranched hollow flowering stems with terminal or spiciform synflorescences (Lipschitz 1979, Chater and Kitamura 1982, Ohba and Akiyama 1992, Shih and Jin 1999, Fujikawa and Ohba 2002). *Eriocoryne* is distributed in the Himalaya, SW China and Central Asia, occurring in the subnival and alpine zones at elevations between 3,000 and 5,500 m.

The majority of the species are extremely curious in appearance. The whole plant or the upper half of the plant is covered with dense, whitish or pinkish wool-like hairs

(Fujikawa 2000, 2002, Ohba and Fujikawa 2000). Their extremely downy nature (Tsukaya and Tsuge 2001) has led to the name “snowball plants” being given to them (Ohba 1988, Ohba and Akiyama 1998). Snowball plants are regarded as being adapted to the harsh environment in the alpine and subnival zones. The dense woolly hairs may protect the primordia or young reproductive shoots from the cold (Ohba 1988, Tsukaya and Tsuge 2001), and also to retain heat on the upper surface of the inflorescence (Tsukaya et al. 2002). It also has been proposed that the woolly hairs might be attractive to pollinators and other insects (Ohba 1988, Tsukaya et al. 2002).

Previous cytological studies of *Saussurea* have concentrated on the species of temperate and subarctic regions of eastern Asia and

Russia. The 103 species previously investigated have shown a wide range of variation in chromosome number, $2n=24, 26, 28, 30, 32, 34, 36, 38, 39, 42, 48, c. 50, 51, 52, 54, 72$ and 78 (see Fedorov 1969, Moore 1973, 1974, 1977, Goldblatt 1981, 1984, 1985, 1988, Goldblatt and Johnson 1990, 1991, 1994, 1996, 1998, 2000). The cytological studies in subgenus *Eriocoryne*, however, have been limited and chromosome numbers have been reported from only ten species (Shetty 1967, Malla et al. 1979, Liu et al. 1994, Huang et al. 1996, Amano and Ohba 2000). There have been no karyological analysis because of the difficulty of obtaining materials in good condition.

This paper reports on the chromosome numbers at the population level for taxa re-

ported previously and on the karyotypes of the Nepal Himalayan species of subgenus *Eriocoryne*. The relationship between cytology and taxonomy is discussed.

Materials and Methods

Materials were collected from twenty populations of eight species belonging to subgenus *Eriocoryne* in their native habitat in the Nepal Himalaya during 1997 and 1999 (Wakabayashi 1998, Fujikawa 2000, Fujikawa and Omori 2000). The collection localities, voucher specimens and chromosome numbers are listed in Table 1.

Chromosome counts were made at somatic metaphase using the squash technique. At least two individuals in each population were analyzed. Shoot meristems collected in

Table 1. Chromosome numbers of *Saussurea* subgenus *Eriocoryne* and the voucher data from Nepal

Species	Chromosome number (2n)	Locality	Voucher specimen
<i>S. gossipiphora</i> D.Don	36	Solukhumbu Distr., Tangnang, 4700 m	Wakabayashi & al. 9730261
	36	Solukhumbu Distr., Khola Kharka-Rahgdu Kharka, 4320 m	Wakabayashi & al. 9730158
	36	Solukhumbu Distr., Khare-Tangnang, 4910 m	Wakabayashi & al. 9730303
	36	Solukhumbu Distr., Bhedi Kharka-Dudh Kund, 4590 m	Wakabayashi & al. 9730329
	36	Solukhumbu Distr., Dudh Kund, 4570 m	Wakabayashi & al. 9730331
	36	Solukhumbu Distr., Thuli Kharka-Kurki, 4100 m	Wakabayashi & al. 9730366
<i>S. laminamaensis</i> Kitam.	36	Sankhuwasabha Distr., Bandhuke, 4400 m	Tateno & al. 9920067
	36	Sankhuwasabha Distr., Jaljale Pokhari-Tin Pokhari, 4200 m	Tateno & al. 9920058
<i>S. nishiokae</i> Kitam.	36	Solukhumbu Distr., Dig Kharka, 4800 m	Wakabayashi & al. 9730259
	36	Solukhumbu Distr., Khare, 4780 m	Wakabayashi & al. 9730301
	36	Solukhumbu Distr., Dudh Kund, 4780 m	Wakabayashi & al. 9730335
<i>S. tridactyla</i> Sch.Bip. ex Hook.f.	36	Solukhumbu Distr., Khare, 4780 m	Wakabayashi & al. 9730298
<i>S. simpsoniana</i> (Field. & Gardn.) Lipsch.	32+0~2B	Solukhumbu Distr., Khare, 4750 m	Wakabayashi & al. 9730278
	32	Mustang Distr., Bhatase Danda, 4540 m	Fujikawa 9920114
	32	Solukhumbu Distr., Tangnang, 4470 m	Wakabayashi & al. 9730236
<i>S. topkegolensis</i> H.Ohba & S.Akiyama	32	Solukhumbu Distr., Dig Kharka, 4700 m	Wakabayashi & al. 9730258
	32	Solukhumbu Distr., Khare, 4820 m	Wakabayashi & al. 9730284
	32	Solukhumbu Distr., Bhedi Kharka-Dudh Kund, 4200 m	Wakabayashi & al. 9730328
<i>S. kanaii</i> K.Fujikawa & H.Ohba	32	Mustang Distr., Bhatase Danda, 4410 m	Fujikawa 9920121
<i>S. bhutkesh</i> K.Fujikawa & H.Ohba	36	Solukhumbu Distr., Dig Kharka, 4770 m	Wakabayashi & al. 9730261

nature were fixed with in Newcomer's fluid (see Sharma and Sharma 1980) in the field after pretreatment with 0.05 % colchicine solution for about 3–4 hours. To macerate the cell walls, the meristems were soaked in a solution of 2 % pectinase and 4 % cellulase (1 : 1) for 20 minutes–3 hours at 37 °C, then cleaned in an alcohol series and distilled water. They were stained with 2 % aceto-orcein for 12–20 hours and squashed.

For karyotype analysis the somatic chromosomes at metaphase were utilized. To determine the chromosome size, five well-spread cells were used (in *S. laminamaensis* three cells were used). The calculations were done for arm ratio (relative length of long/short arms) and total chromosome length (TCL). Cytological terminology follows Levan et al. (1964) for centromere position as follows: median (m), submedian (sm), subterminal (st) and terminal (t).

Voucher specimens for this study are deposited in the Herbarium of the University of Tokyo (TI).

Results and Comments

The chromosome numbers of the eight species of subgenus *Eriocoryne* are shown in Table 1. The somatic chromosomes of each species are illustrated in Fig. 1. The karyotypes of the seven species are presented in Fig. 2. The chromosome number and karyotype of each species are described below.

Saussurea gossipiphora D. Don

The chromosome counts of individuals from seven populations in the Nepal Himalaya were $2n = 36$ (Fig. 1, A, Table 1). These findings agree with a previous report of $2n = 36$ by Amano and Ohba (2000), but differs from the $n = 13$ report by Malla et al. (1979). The number ' $n = 13$ ' appears to be a misprint for $n = 18$. The karyotype comprised 26 median and submedian centromeric chromosomes and 10 subterminal and

terminal centromeric chromosomes (Fig. 2, A). The chromosomes decreased in length gradually from 6.1 μm to 1.5 μm . Total chromosome length was about 101.0 μm , with the longest/shortest ratio, 4.1.

Saussurea laminamaensis Kitam.

The somatic chromosome number was $2n = 36$ (Fig. 1, B, Table 1). The chromosome number of this species is reported for the first time. The karyotype consisted of 24 median and submedian centromeric chromosomes and 12 subterminal and terminal centromeric chromosomes (Fig. 2, B). The chromosomes gradually decreased in length from 6.5 μm to 1.6 μm . Total length was about 109.6 μm , with the longest/shortest ratio, 4.1.

Saussurea nishiokae Kitam.

The somatic chromosome number from individuals from three populations was $2n = 36$ (Fig. 1, C, Table 1). This count agrees with the report by Amano and Ohba (2000). The karyotype consisted of 22 median and submedian centromeric chromosomes and 14 subterminal and terminal centromeric chromosomes (Fig. 2, C). The chromosomes gradually decreased in length from 6.0 μm to 1.6 μm . Total chromosome length was about 112.3 μm , with the longest/shortest ratio, 3.8.

Saussurea tridactyla Sch. Bip. ex Hook. f.

The somatic chromosome number was $2n = 36$ (Fig. 1, D, Table 1). Our observations agree with a previous report by Amano and Ohba (2000). The karyotype comprised 22 median and submedian centromeric chromosomes and 14 subterminal and terminal centromeric chromosomes (Fig. 2, D). The chromosomes decreased in length gradually from 6.3 μm to 1.7 μm . Total chromosome length was about 105.3 μm , with the longest/shortest ratio, 3.7.

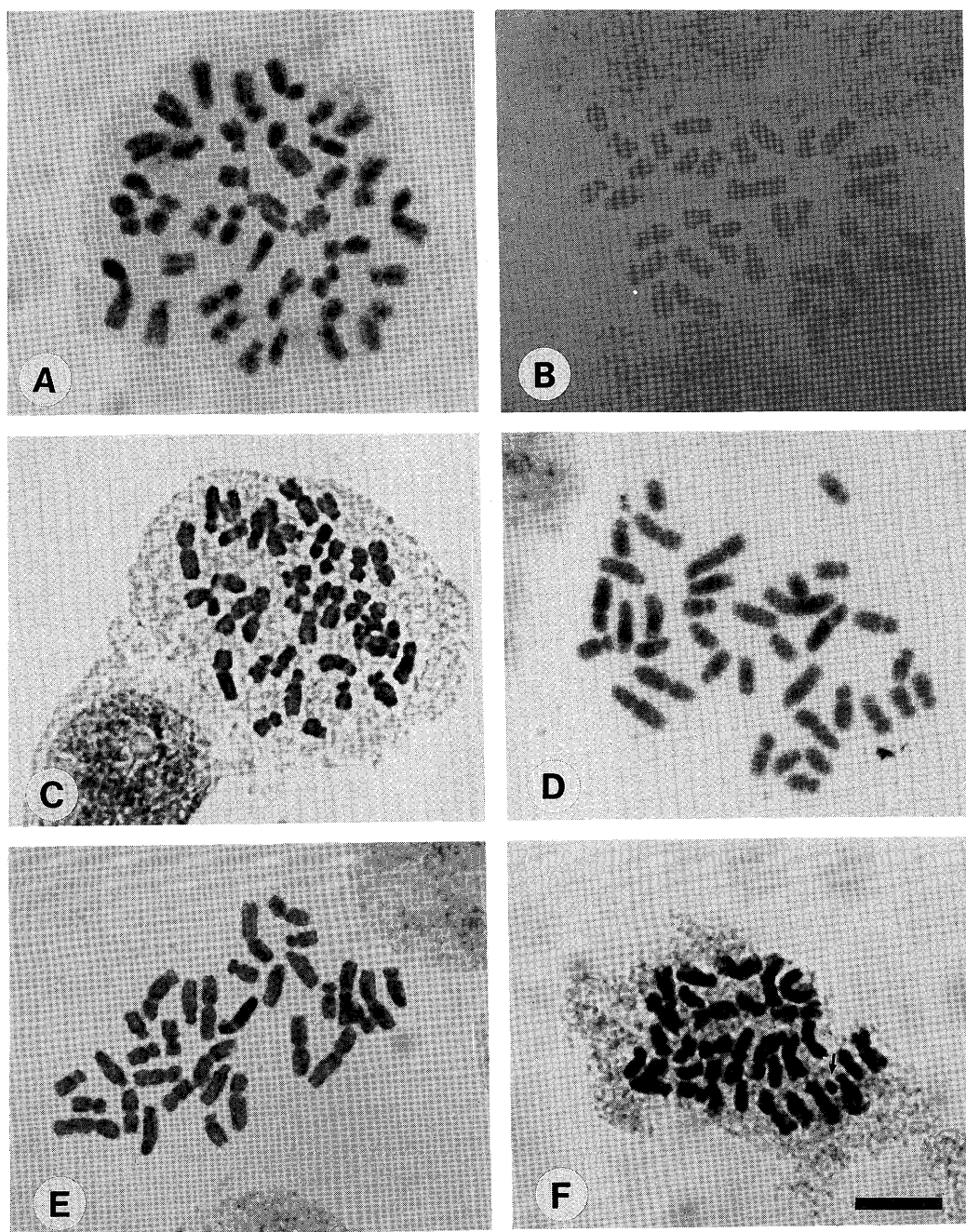


Fig. 1. Somatic chromosomes of the eight species of *Saussurea* subgenus *Eriocoryne*. A: *Saussurea gossipiphora*. B: *S. laminamaensis*. C: *S. nishiokae*. D: *S. tridactyla*. E, F: *S. simpsoniana*. Bar = 10 μ m. Arrow indicates B-chromosome.

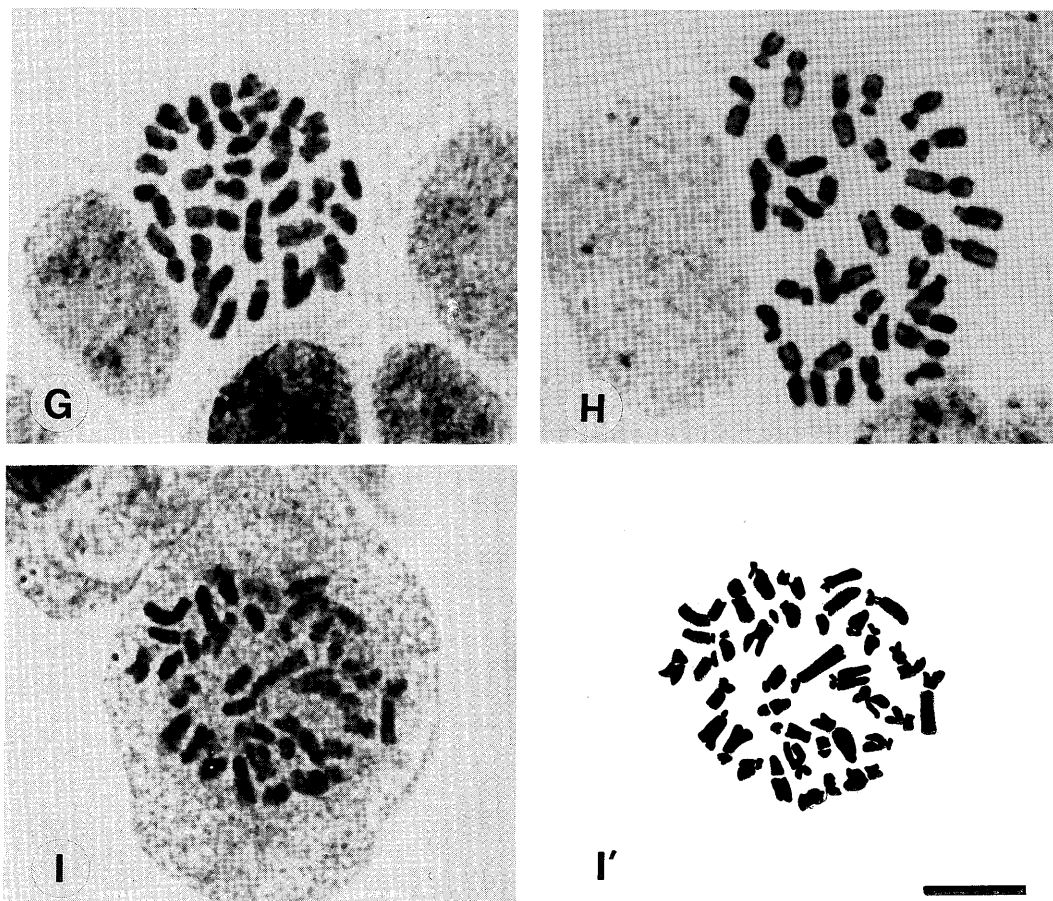


Fig. 1. Continued. G: *Saussurea topkegolensis*. H: *S. kanaii*. I: *S. bhutkesh*. I': diagram of *S. bhutkesh*. Bar = 10 μ m.

Saussurea simpsoniana (Field & Gardner) Lipsch.

The somatic chromosome numbers of individuals from two populations were $2n = 32$ with or without one or two B-chromosomes (Fig. 1, E, F, Table 1). Our findings confirm previous counts of $n = \text{ca. } 16$ reported by Shetty (1967, as *S. sacra* Edgew.) and of $2n = 32$ by Amano and Ohba (2000). The karyotype comprised 22 median and submedian centromeric chromosomes and 10 subterminal and terminal centromeric chromosomes (Fig. 2, E). The chromosomes decreased in length gradually from 5.9 μ m to

1.4 μ m. Total chromosome length was about 110.8 μ m, with the longest/shortest ratio, 4.2.

Saussurea topkegolensis H. Ohba & S. Akiyama

The somatic chromosome number in individuals from four populations was $2n = 32$ (Fig. 1, G, Table 1). Our observations support the previous report by Amano and Ohba (2000). The karyotype comprised 22 median and submedian centromeric chromosomes and 10 subterminal and terminal centromeric chromosomes (Fig. 2, F). The chromosomes gradually decreased in length from 5.8 μ m to

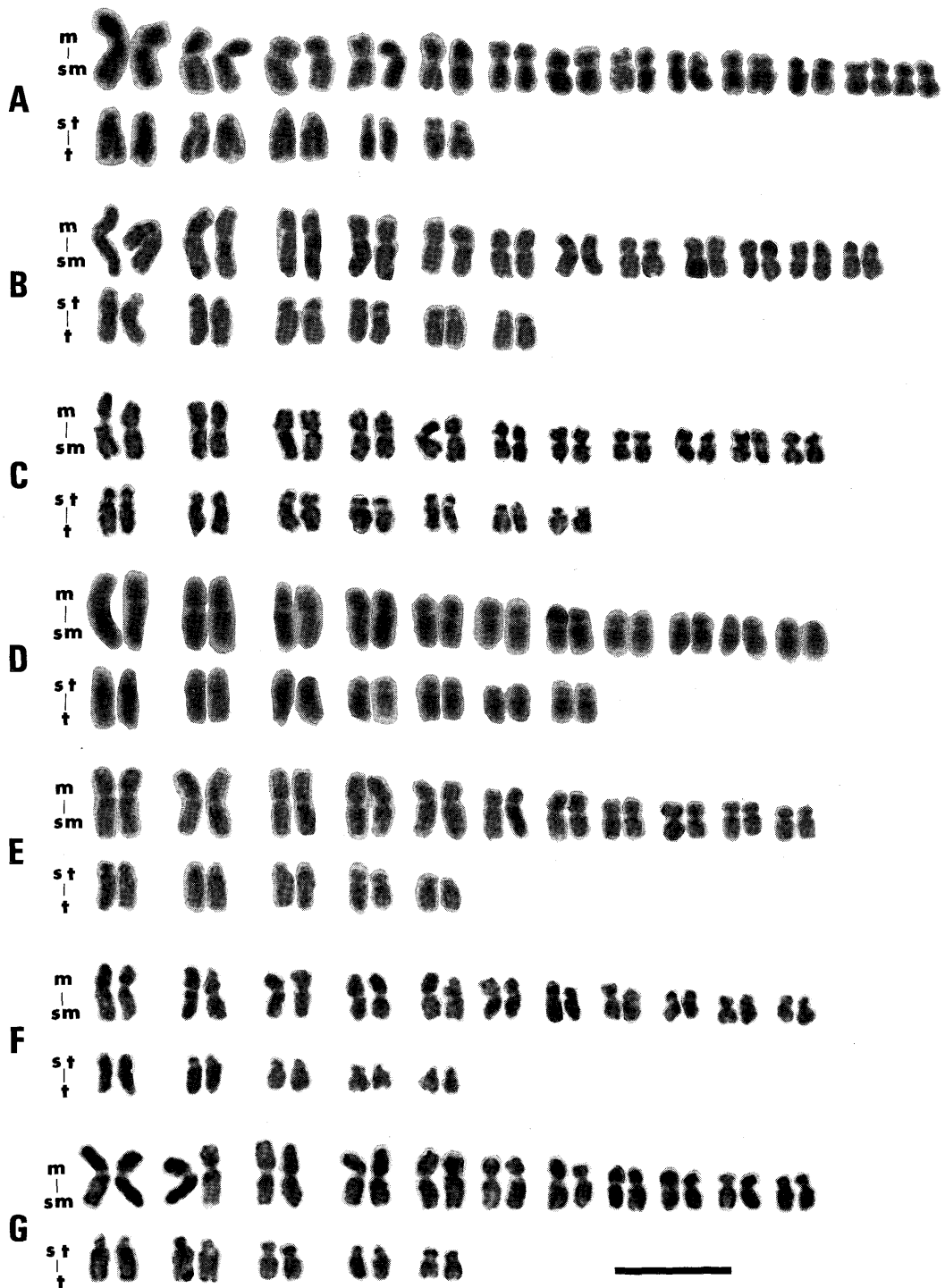


Fig. 2. Karyotypes of the seven species of *Saussurea* subgenus *Eriocoryne*. A: *Saussurea gossipiphora*. B: *S. laminamaensis*. C: *S. nishiokae*. D: *S. tridactyla*. E: *S. simpsoniana*. F: *S. topkegolensis*. G: *S. kanaii*. Bar = 10 μ m.

1.6 μm . Total chromosome length was about 104.4 μm , with the longest/shortest ratio, 3.6.

Saussurea kanaii K.Fujikawa & H.Ohba

The somatic chromosome number was $2n = 32$ (Fig. 1, H, Table 1). The chromosome number of this species is reported for the first time. The karyotype comprised 22 median and submedian centromeric chromosomes and 10 subterminal and terminal centromeric chromosomes (Fig. 2, G). The chromosomes gradually varied from 5.7 μm to 1.8 μm , with total chromosome length about 108.7 μm , and the longest/shortest ratio, 3.2.

Saussurea bhutkesh K.Fujikawa & H.Ohba

The somatic chromosome number of $2n = 36$ (Fig. 1, I, I', Table 1) is the first report for this species. Karyotype analysis was not made, since the chromosomes were at prometaphase.

Discussion

Among the eight species of subgenus *Eriocoryne*, the somatic chromosome number $2n = 32$ was found in three species and $2n = 36$ in five species. These findings agree with previous cytological studies of the Himalayan species of subgenus *Eriocoryne* (Shetty 1967, Amano and Ohba 2000). Considering previous chromosome counts in *Saussurea*, these are all diploid with two basic numbers, $x = 16$ and 18. Within the Himalaya, the species of *Eriocoryne* show a wide range of morphological diversity (Hooker 1881, Kitamura 1969, Ohba 1988). Chromosome counts and karyotype analysis show that speciation has occurred at the diploid level, accompanied by formation of aneuploid.

In contrast, chromosome numbers of $2n = 28$, 30 and 48 were reported for Chinese species of *Eriocoryne* by Liu et al. (1994) and Huang et al. (1996). Those numbers have never been reported from Himalayan

Eriocoryne. Liu et al. (1994) reported the chromosome number of *Saussurea thoroldii* Hemsl. from Tibet to be $2n = 30$. But the photograph of the chromosome complement in their paper appears to show 32 chromosomes. Huang et al. (1996) reported chromosome counts for *S. medusa* Maxim., *S. paxiana* Diels and *S. wellbyi* Maxim. as $2n = 28$ and for *S. gnaphalodes* (Royle) Sch.Bip. as $2n = 48$. Those numbers are ambiguous, however, since the chromosomes in their photographs are unclear. Further cytological studies are needed to confirm the chromosome numbers in *Eriocoryne*, especially for the species within China.

Our karyological analysis shows that all seven species that we examined share a similar pattern, although some differences in total chromosome length and karyotype formula can be seen. The karyotype of all seven species consisted of median, submedian, subterminal and terminal centromeric chromosomes, and the size of chromosomes decreased in a continuous series from the longest to the shortest pair. The chromosome complement was $2n = 22$ (m + sm) + 10 (st + t) in all the species with $2n = 32$. Among the species with $2n = 32$, the total length of the chromosomes in *S. simpsoniana* was the highest (TCL = ca. 110.8 μm) and the shortest total length was in *S. topkegolensis* (TCL = ca. 104.4 μm). Amano and Ohba (2000) pointed out that *S. topkegolensis* needed to be compared with *S. simpsoniana* phylogenetically. Our study also suggests similarities in their karyotypes. Our cytological results do not support a close relationship between *S. topkegolensis* and *S. gossipiphora*, which was considered a possibility based on vegetative characters by Ohba and Akiyama (1992). We also have found morphological differences between them. *Saussurea gossipiphora* has 3 to 6 series of involucrellary phyllaries, with the innermost phyllaries approximately twice as long as the outermost ones, the sessile capitula densely crowded on

the flat to convex stem apex, and the synflorescence concealed by the wool-like hairs at least until flowering time. *Saussurea topkegolensis* has 3 or 4 series of involucre phyllaries with the innermost phyllaries nearly equaling the outermost in length, very shortly pedunculate capitula arranged loosely on the obovoid stem apex and half of the capitula exerted beyond the woolly hairs covering the stem apex at flowering time. Additionally, we recently found that *S. simpsoniana*, *S. topkegolensis* and *S. kanaii* commonly have 3 or 4 imbricate involucre phyllaries, the setae of the receptacle 1.2 to 2.0 mm long, with or without sparse club-shaped outgrowths on the upper part of the achene and half of capitula or synflorescence exerted beyond the wool-like hairs at flowering time, in addition to their being clearly distinguished by the shape of the involucre, serration of the cauline leaves and reproductive pattern (e.g., Fujikawa 2002, Fujikawa and Ohba 2002). The karyotype similarities therefore appear to correlate with the morphological similarities.

In contrast, differences in chromosome complement can be recognized among the species with $2n = 36$. The complement having five pairs of st-t type was found in *Saussurea gossipiphora*, the complement with six pairs of st-t type was found in *S. laminamaensis* and the complement with seven pairs of st-t type was found in *S. nishiokae* and *S. tridactyla*. Among the species with $2n = 36$ the longest total length of the chromosomes was observed in *S. nishiokae* (TCL = c. 112.3 μm), while the lowest values were found in *S. gossipiphora* (TCL = c. 101.0 μm). Those species are distinct in their morphology (Kitamura 1969, 1981, Fujikawa 2002, Fujikawa and Ohba 2002). Their karyotype differences therefore seem to be correlated with their morphological differences.

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Endnote

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藤川和美¹, 大場秀章: ネパール・ヒマラヤ地域
に産するトウヒレン属 *Eriocoryne* 亜属植物 (キク
科) の細胞学的研究

ネパールヒマラヤ地域に産するトウヒレン属
Eriocoryne 亜属 (キク科) 8 種について20集団か
ら採集した植物を用いて細胞学的研究を行った。
染色体数は *Saussurea gossipiphora*, *S. laminamaen-*
sis, *S. nishiokae*, *S. tridactyla*, *S. bhutkesh* の5種で
 $2n = 36$, *S. simpsoniana*, *S. topkegolensis*, *S. kanaii*
の3種で $2n = 32$ が見いだされた。 *S. laminamaen-*
sis, *S. kanaii*, *S. bhutkesh* の染色体数の報告は今回
が初めてである。本研究により得られたこれらの
種の染色体数はすべて2倍体であり、基本数はそ
れぞれ $x = 16, 18$ と推定される。そして *Eriocoryne*
亜属は異数性をともない形態の多様化が起きたこ
とが示唆される。また *S. bhutkesh* を除く7種で

核型分析を行った。その結果、核形態は共通して
中部, 次中部, 次端部, 端部動原体をもつ染色体
から構成され, それらは一番長い染色体から短い
ものへ徐々に勾配的に長さが減少した。核型は染
色体数が $2n = 32$ である *S. simpsoniana*, *S. topkego-*
lensis, *S. kanaii* 3 種は同じ $2n = 22 (m + sm) + 10$
(st+t) であった。染色体数が $2n = 36$ である種は
それぞれ *S. gossipiphora* が $2n = 26 (m + sm) + 10$
(st + t), *S. laminamaensis* が $2n = 24 (m + sm) + 12$
(st + t), *S. nishiokae* と *S. tridactyla* が $2n = 22 (m +$
 $sm) + 14 (st + t)$ であった。 *Eriocoryne* 亜属の核型
解析は本論文が初めての発表である。

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